51320/JPW/AKC

Application for United States Letters Patent

To all whom it may concern:

Be it known that Graham P. Allaway, Tatjana Dragic, Virginia M. Litwin, Paul J. Maddon and John P. Moore

have invented certain new and useful improvements in

USES OF A CHEMOKINE RECEPTOR FOR INHIBITING HIV-1 INFECTION

of which the following is a full, clear and exact description.

Applicants: Olson and Maddon
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Exhibit 9

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USES OF A CHEMOKINE RECEPTOR FOR INHIBITING HIV-1 INFECTION

The invention described in this application was made with support under Grants Nos. Al35522, Al36057, Al36082 and Al38573 from the National Institutes of Health, U.S. Department of Health and Human Service. Accordingly, the United States Government has certain rights in this invention.

Throughout this application, various references are referred to by arabic numerals within parenthesis. Disclosures of these publications in their entireties are hereby incorporated by reference into this application to more fully describe the state of the art to which this invention pertains. Full bibliographic citation for these references may be found immediately preceding the claims.

Background of the Invention

The replication of primary, non-syncytium-inducing (NSI) HIV-1 isolates in $CD4^+$ T-cells is inhibited by the C-C ß-chemokines MIP-1α, MIP-1ß and RANTES (1,2), but T-cell line-adapted (TCLA) or syncytium-inducing (SI) primary strains are insensitive (2,3). The ß-chemokines are small (8kDa), related proteins active on cells of the lymphoid and monocyte lineage (4-8). Their receptors are members of the 7-membrane-spanning, G-protein-linked superfamily, one of which (the LESTR orphan receptor) has been identified as the second receptor for TCLA HIV-1 strains, and is now designated fusin (9). Fusin is not known to be a ß-chemokine receptor (7-9).

Summary of the Invention

This invention provides a polypeptide having a sequence corresponding to the sequence of a portion of a chemokine receptor capable of inhibiting the fusion of HIV-1 to $CD4^+$ cells and thus infection of the cells. In an embodiment, the chemokine receptor is C-C CKR-5. In another embodiment, the polypeptide comprises amino acids having a sequence of at least one extracellular domain of C-C CKR-5.

In a preferred embodiment, the portion of a chemokine receptor comprises amino acid sequence MDYQVSSPIYDINYYTSEPCQKINVKQIAAR (SEQ ID NO: 5). In another preferred embodiment, the portion comprises amino acid sequence HYAAAQWDFGNTMCQ (SEQ ID NO: 6). In still another preferred embodiment, the portion comprises amino acid sequence RSQKEGLHYTCSSHFPYSQYQFWKNFQTLKIV (SEQ ID NO: 7). In a separate preferred embodiment, the portion comprises amino acid sequence QEFFGLNNCSSSNRLDQ (SEQ ID NO: 8). The portion of the chemokine receptor C-C CKR-5 may comprise one or more of the above sequences. The polypeptides may contain part of the above illustrated sequences and still be capable of inhibiting HIV-1 infection. The minimal number of amino acids sufficient to inhibit HIV-1 infection may be determined by the RET or infection assays as described below.

This invention also provides a pharmaceutical composition comprising effective amount of one or more of the above polypeptides and a pharmaceutically acceptable carrier.

This invention also provides a polypeptide having a sequence corresponding to that of a portion of an HIV-1 glycoprotein capable of specifically binding to the chemokine receptor C-C CKR-5.

This invention provides a pharmaceutical composition comprising effective amount of one of more polypeptides having a sequence corresponding to the sequence of a portion of an HIV-1 glycoprotein capable of specifically binding to the chemokine receptor C-C CKR-5 and a pharmaceutically acceptable carrier.

This invention provides an antibody or a portion of an antibody capable of binding to a chemokine receptor on a $CD4^+$ cell and inhibiting HIV-1 infection of the cell.

This invention also provides a pharmaceutical composition comprising an effective amount of an antibody capable of binding to a chemokine receptor on a $CD4^+$ cell and inhibiting HIV-1 infection of the cell and a pharmaceutically acceptable carrier.

This invention provides a method of treating an HIV-1 infected subject comprising administering to the subject the above polypeptides, antibody and pharmaceutical compositions.

This invention provides a method of reducing the likelihood of a subject from becoming infected by HIV-1 comprising administering to the subject the above pharmaceutical compositions.

This invention provides a method for inhibiting fusion of HIV-1 to $CD4^+$ cells which comprises contacting $CD4^+$ cells with a non-chemokine agent capable of binding to the chemokine receptor C-C CKR-5 in an amount and under conditions such that fusion of HIV-1 to the $CD4^+$ cells is inhibited.

This invention provides a method for inhibiting HIV-1

infection of $CD4^+$ cells which comprises contacting $CD4^+$ cells with a non-chemokine agent capable of binding to the chemokine receptor C-C CKR-5 in an amount and under conditions such that fusion of HIV-1 to the $CD4^+$ cells is inhibited, thereby inhibiting HIV-1 infection of the cells.

This invention provides a non-chemokine agent capable of binding to the chemokine receptor C-C CKR-5 and inhibiting HIV-1 infection.

This invention provides a molecule capable of binding to the chemokine receptor C-C CKR-5 and inhibiting HIV-1 infection comprising a non-chemokine agent linked to a ligand capable of binding to a cell surface receptor of the $CD4^+$ cells other than the chemokine receptor such that the binding of the non-chemokine agent to the chemokine receptor does not prevent the binding of the ligand to the other receptor.

This invention provides a pharmaceutical composition comprising an amount of the above molecules effective to inhibit fusion of HIV-1 to $CD4^+$ cells and a pharmaceutically acceptable carrier.

This invention also provides a molecule capable of binding to the chemokine receptor C-C CKR-5 and inhibiting fusion of HIV-1 to $CD4^+$ cells comprising a non-chemokine agent linked to a compound capable of increasing the *in vivo* half-life of the non-chemokine agent.

This invention further provides a pharmaceutical composition comprising an amount of the molecule capable of binding to the chemokine receptor C-C CKR-5 and inhibiting HIV-1 infection comprising a non-chemokine agent linked to a compound capable of increasing the *in vivo* half-life of the non-chemokine agent effective to inhibit fusion of HIV-1 to

$CD4^+$ cells and a pharmaceutically acceptable carrier.

This invention provides a method for reducing the likelihood of HIV-1 infection in a subject comprising administering the above pharmaceutical compositions to the subject.

This invention provides a method for treating HIV-1 infection in a subject comprising administering the above pharmaceutical compositions to the subject.

This invention provides a method for determining whether a non-chemokine agent is capable of inhibiting the fusion of HIV-1 to a $CD4^+$, C-C $CKR-5^+$ cell which comprises: (a) contacting a $CD4^+$, C-C $CKR-5^+$ cell, which is labeled with a first dye, with a cell expressing an appropriate HIV-1 envelope glycoprotein on its surface, which is labeled with a second dye, in the presence of excess of the agent under conditions permitting the fusion of the $CD4^+$ and C-C $CKR-5^+$ cell to the cell expressing the HIV-1 envelope glycoprotein on its surface in the absence of an agent known to inhibit fusion of HIV-1 to $CD4^+$, C-C $CKR-5^+$ cell, the first and second dyes being selected so as to allow resonance energy transfer between the dyes; (b) exposing the product of step (a) to conditions which would result in resonance energy transfer if fusion has occurred; and (c) determining whether there is resonance energy transfer, the absence or reduction of transfer indicating that the agent is capable of inhibiting fusion of HIV-1 to $CD4^+$ and C-C $CKR-5^+$ cells.

This invention also provides a transgenic nonhuman animal which comprises an isolated DNA molecule encoding the chemokine receptor C-C CKR-5. In an embodiment, this transgenic nonhuman animal further comprises an isolated DNA molecule encoding a sufficient portion of the CD4 molecule to permit binding the HIV-1 envelope glycoprotein.

This invention further provides a transgenic nonhuman animal which comprises an isolated DNA molecule encoding the chemokine receptor C-C CKR-5 and an isolated DNA molecule encoding fusin. In an embodiment, this transgenic nonhuman animal further comprises an isolated DNA molecule encoding the full-length or portion of the CD4 molecule sufficient for binding the HIV-1 envelope glycoprotein.

Finally, this invention provides transformed cells which comprise an isolated nucleic acid molecule encoding the chemokine receptor C-C CKR-5.

Description of the Figures

Figure 1: Specificity. time course and stage of ß-chemokine inhibition of HIV-1 replication

(a) PM1 cells (1 x10^6) were preincubated with RANTES + MIP- 1α + MIP-1ß (R/Mα/Mß; 100ng/ml of each) for 24h (-24h) or 2h (-2h), then washed twice with phosphate buffered saline (PBS). HIV-1 (BaL env-complemented) virus (50ng of p24; see legend to Table 1) was added for 2h, then the cells were washed and incubated for 48h before measurement of luciferase activity in cell lysates as described previously (10,11). Alternatively, virus and R/Mα/Mß were added simultaneously to cells, and at the indicated time points (1h, 3h, etc) the cells were washed twice in PBS, resuspended in culture medium and incubated for 48h prior to luciferase assay. Time 0 represents the positive control, to which no ß-chemokines were added. +2h represents the mixture of virus with cells for 2h prior to washing twice in PBS, addition of R/Mα/Mß and continuation of the culture for a further 48h before luciferase assay.

(b) PM1 cells (1x10^6) were infected with HIV-1 (500pg p24) grown in CEM cells (NL4/3; lanes 1-4) or macrophages (ADA; lanes 5-8), in the presence of 500ng/ml of RANTES (lanes 1 and 5) or MIP-1ß (lanes 2 and 6), or with no ß-chemokine (lanes 4 and 8). Lanes 3 and 7 are negative controls (no virus). All viral stocks used for the PCR assay were treated with DNAse for 30 min at 37°C, and tested for DNA contamination before use. After

2h, the cells were washed and resuspended in medium containing the same ß-chemokines for a further 8h. DNA was then extracted from infected cells using a DNA/RNA isolation kit (US Biochemicals). First round nested PCR was performed with primers: U3+, 5'-CAAGGCTACTTCCCTGATTGGCAGAACTACACACCAGG-3' (SEQ ID NO:1) preGag, 5'-AGCAAGCCGAGTCCTGCGTCGAGAG-3' (SEQ ID NO:2) and the second round with primers: LTR-test, 5'-GGGACTTTCCGCTGGGGACTTTC 3' (SEQ ID NO :3) LRC2, 5'-CCTGTTCGGGCGCCACTGCTAGAGATTTTCCAC 3' (SEQ ID NO:4) in a Perkin Elmer 2400 cycler with the following amplification cycles: 94°C for 5 min, 35 cycles of 94°C for 30s, 55°C for 30s, 72°C for 30s, 72°C for 7 min. M indicates 1kb DNA ladder; 1, 10, 100, 1000 indicate number of reference plasmid (pAD8) copies. The assay can detect 100 copies of reverse transcripts.

Figure 2: <u>HIV-1 env-mediated membrane fusion of cells transiently expressing C-C CKR-5</u>

Membrane fusion mediated by ß-chemokine receptors expressed in HeLa cells was demonstrated as follows: Cells were transfected with control plasmid pcDNA3.1 or plasmid pcDNA3.1 -CKR constructs using lipofectin (Gibco BRL). The pcDNA3.1 plasmid carries a T7-polymerase promoter and transient expression of ß-chemokine receptors was boosted by infecting cells with $1x10^7$ pfu of vaccinia encoding the T7-polymerase (vFT7.3) 4h post-lipofection (9). Cells were then cultured overnight in R18-containing media and were tested for their ability to fuse with HeLa-JR-FL cells (filled columns) or HeLa-BRU cells (hatched column) in the RET assay. The %RET with control

HeLa cells was between 3% and 4% irrespective of the transfected plasmid.

Detailed Description of the Invention

This invention provides a polypeptide having a sequence corresponding to the sequence of a portion of a chemokine receptor capable of inhibiting the fusion of HIV-1 to $CD4^+$ cells and thus infection of the cell. In an embodiment, the chemokine receptor is C-C CKR-5. In another embodiment, the fragment comprises at least one extracellular domain of the chemokine receptor C-C CKR-5.

The sequence of a portion of the chemokine receptor includes the original amino acids or modified amino acids from the receptor, their derivatives and analogues. Such sequence should retain the ability to inhibit HIV-1 infection. Sequences of fusin are also included.

In a preferred embodiment, the portion of a chemokine receptor comprises amino acid sequence MDYQVSSPIYDINYYTSEPCQKINVKQIAAR (SEQ ID NO: 5). In another preferred embodiment, the portion comprises amino acid sequence HYAAAQWDFGNTMCQ (SEQ ID NO: 6). In still another preferred embodiment, the portion comprises amino acid sequence RSQKEGLHYTCSSHFPYSQYQFWKNFQTLKIV (SEQ ID NO: 7). In a separate preferred embodiment, the portion comprises amino acid sequence QEFFGLNNCSSSNRLDQ (SEQ ID NO: 8). The portion of the chemokine receptor C-C CKR-5 may comprise one or more of the above sequences. The polypeptides may contain part of the above illustrated sequences and still be capable of inhibiting HIV-1 infection. The minimal number of amino acids sufficient to inhibit HIV-1 infection may be determined by the RET or infection assays as described below.

The polypeptides described above may be fusion molecules such that the fragments are linked to other molecules. In

an embodiment, the molecule is a CD4-based molecule. CD4-based molecules are known in the art and described in Allaway et al. (1996), Patent Cooperation Treaty Application No. PCT/US95/08805, publication no. WO 96/02575, the content of which is incorporated by reference into this application. In another embodiment, the polypeptide contains multiple units of one or more portions of a chemokine receptor. In a preferred embodiment, the polypeptide contains sequences corresponding to multiple units of one or more extracellular domains of the chemokine receptor C-C CKR-5.

This invention also provides a pharmaceutical composition comprising effective amount of the above polypeptide and a pharmaceutically acceptable carrier.

As used herein, the term "pharmaceutically acceptable carrier" encompasses any of the standard pharmaceutical carriers, such as a phosphate buffered saline solution, water, and emulsions, such as an oil/water or water/oil emulsion, and various types of wetting agents.

This invention also provides a polypeptide having a sequence corresponding to that of a portion of an HIV-1 envelope glycoprotein capable of specifically binding to the chemoreceptor C-C CKR-5. Such a sequence may be identified by routine experiments. For example, overlapping synthetic peptides representing fragments of gp120 or gp41 can be tested in the RET assay for their ability to inhibit fusion between cells expressing the envelope glycoprotein of HIV-1 clinical isolates and cells expressing CD4 and C-C CKR-5. Peptides inhibiting fusion in this assay are also screened in the RET assay for ability to inhibit fusion mediated by the envelope glycoprotein of HIV-1 laboratory-adapted-strains and peptides which are inhibitory in this later assay are discarded. As an alternative method, the peptides

can be tested for their ability to compete with chemokines for binding to cell expressing C-C CKR-5.

This invention provides a pharmaceutical composition comprising effective amount of the polypeptide comprising a fragment of HIV-1 glycoprotein capable of specifically binding to the chemokine receptor C-C CKR-5 and a pharmaceutically acceptable carrier.

This invention provides an antibody or a portion of an antibody thereof capable of binding to a chemokine receptor on a $CD4^+$ cell and inhibiting HIV-1 infection of the cell.

This invention also provides a pharmaceutical composition comprising effective amount of antibody capable of binding to a chemokine receptor and inhibiting HIV-1 infection and a pharmaceutically acceptable carrier.

This invention provides a method of treating an HIV-1 infected subject comprising administering to the subject the above pharmaceutical compositions.

This invention provides a method of reducing the likelihood of a subject from becoming infected by HIV-1 comprising administering to the subject the above pharmaceutical compositions.

This invention provides a method for inhibiting fusion of HIV-1 to $CD4^+$ cells which comprises contacting $CD4^+$ cells with a non-chemokine agent capable of binding to the chemokine receptor C-C CKR-5 in an amount and under conditions such that fusion of HIV-1 to the $CD4^+$ cells is inhibited.

This invention provides a method for inhibiting HIV-1

infection of $CD4^+$ cells which comprises contacting $CD4^+$ cells with a non-chemokine agent capable of binding to the chemokine receptor C-C CKR-5 in an amount and under conditions such that fusion of HIV-1 to the $CD4^+$ cells is inhibited, thereby inhibiting HIV-1 infection.

The non-chemokine agents of this invention are capable of binding to chemokine receptors and inhibiting fusion of HIV-1 to $CD4^+$ cells. The non-chemokine agents include, but are not limited to, chemokine fragments and chemokine derivatives and analogues, but do not include naturally occurring chemokines.

In an embodiment, the non-chemokine agent is an oligopeptide. In another embodiment, the non-chemokine agent is a polypeptide. In still another embodiment, the non-chemokine agent is a nonpeptidyl agent.

This invention provides a non-chemokine agent capable of binding to the chemokine receptor C-C CKR-5 and inhibiting fusion of HIV-1 to $CD4^+$ cells.

This invention provides a molecule capable of binding to the chemokine receptor C-C CKR-5 and inhibiting fusion of HIV-1 to $CD4^+$ cells comprising a non-chemokine agent linked to a ligand capable of binding to a cell surface receptor of the $CD4^+$ cells other than the chemokine receptor such that the binding of the non-chemokine agent to the chemokine receptor does not prevent the binding of the ligand to the other receptor. In an embodiment, the cell surface receptor is CD4. In another embodiment, the ligand comprises an antibody or a portion of an antibody.

This invention provides a pharmaceutical composition comprising an amount of the above molecule effective to

inhibit fusion of HIV-1 to $CD4^+$ cells and a pharmaceutically acceptable carrier.

This invention also provides a molecule capable of binding to the chemokine receptor C-C CKR-5 and inhibiting fusion of HIV-1 to $CD4^+$ cells comprising a non-chemokine agent linked to a compound capable of increasing the *in vivo* half-life of the non-chemokine agent. In an embodiment, the compound is polyethylene glycol.

This invention further provides a pharmaceutical composition comprising an amount of the molecule capable of binding to the chemokine receptor C-C CKR-5 and inhibiting fusion of HIV-1 to $CD4^+$ cells comprising a non-chemokine agent linked to a compound capable of increasing the *in vivo* half-life of the non-chemokine agent effective to inhibit fusion of HIV-1 to $CD4^+$ cells and a pharmaceutically acceptable carrier.

This invention provides a method for reducing the likelihood of HIV-1 infection in a subject comprising administering the above pharmaceutical compositions to the subject.

This invention provides a method for treating HIV-1 infection in a subject comprising administering the above pharmaceutical compositions to the subject.

This invention provides a method for determining whether a non-chemokine agent is capable of inhibiting the fusion of HIV-1 to a $CD4^+$, C-C $CKR\text{-}5^+$ cell which comprises: (a) contacting a $CD4^+$, C-C $CKR\text{-}5^+$ cell, which is labeled with a first dye, with a cell expressing an appropriate HIV-1 envelope glycoprotein on its surface, which is labeled with a second dye, in the presence of excess of the agent under conditions permitting the fusion of the $CD4^+$ and C-C $CKR\text{-}5^+$ cell to the cell expressing the HIV-1 envelope glycoprotein

on its surface in the absence of an agent known to inhibit fusion of HIV-1 to $CD4^+$, C-C $CKR-5^+$ cell, the first and second dyes being selected so as to allow resonance energy transfer between the dyes; (b) exposing the product of step (a) to conditions which would result in resonance energy transfer if fusion has occurred; and (c) determining whether there is resonance energy transfer, the absence or reduction of transfer indicating that the agent is capable of inhibiting fusion of HIV-1 to $CD4^+$ and C-C $CKR-5^+$ cells. In an embodiment, the agent is an oligopeptide. In another embodiment, the agent is a polypeptide. In still another embodiment, the agent is a nonpeptidyl agent. In a further embodiment, the $CD4^+$ cell is a PM1 cell. In a separate embodiment, the cell expressing the HIV-1 envelope glycoprotein is a HeLa cell expressing $HIV\text{-}1_{JR\text{-}FL}$ gp120/gp41.

This invention also provides a transgenic nonhuman animal which comprises an isolated DNA molecule encoding the chemokine receptor C-C CKR-5. In an embodiment, this transgenic nonhuman animal further comprises an isolated DNA molecule encoding a sufficient portion of the CD4 molecule to permit binding the HIV-1 envelope glycoprotein.

This invention further provides a transgenic nonhuman animal which comprises an isolated DNA molecule encoding the chemokine receptor C-C CKR-5 and an isolated DNA molecule encoding fusin. In an embodiment, this transgenic nonhuman animal further comprises an isolated DNA molecule encoding a sufficient portion of the CD4 molecule to permit binding the HIV-1 envelope glycoprotein.

One means available for producing a transgenic animal, with a mouse as an example, is as follows: Female mice are mated, and the resulting fertilized eggs are dissected out of their oviducts. The eggs are stored in an appropriate

medium such as M2 medium (Hogan B. et al. Manipulating the Mouse Embryo, A Laboratory Manual, Cold Spring Harbor Laboratory (1986)). DNA or cDNA encoding the C-C CKR-5 chemokine receptor or CD4 is purified from a vector by methods well known in the art. Inducible promoters may be fused with the coding region of the DNA to provide an experimental means to regulate expression of the trans-gene. Alternatively or in addition, tissue specific regulatory elements may be fused with the coding region to permit tissue-specific expression of the trans-gene. The DNA, in an appropriately buffered solution, is put into a microinjection needle (which may be made from capillary tubing using a pipet puller) and the egg to be injected is put in a depression slide. The needle is inserted into the pronucleus of the egg, and the DNA solution is injected. The injected egg is then transferred into the oviduct of a pseudopregnant mouse (a mouse stimulated by the appropriate hormones to maintain pregnancy but which is not actually pregnant), where it proceeds to the uterus, implants, and develops to term. As noted above, microinjection is not the only method for inserting DNA into the egg cell, and is used here only for exemplary purposes.

Finally, this invention provides transformed cells which comprise an isolated nucleic acid molecule encoding the chemokine receptor C-C CKR-5.

The invention will be better understood by reference to the Experimental Details which follow, but those skilled in the art will readily appreciate that the specific experiments detailed are only illustrative, and are not meant to limit the invention as described herein, which is defined by the claims which follow thereafter.

Experimental Details

To study how ß-chemokines inhibit HIV-1 replication, a virus entry assay based on single-cycle infection by an env-deficient virus, NL4/3Δenv (which also carries the luciferase reporter gene), complemented by envelope glycoproteins expressed in trans was used (10,11). Various env-complemented viruses were tested in PM1 cells, a variant of HUT-78 that has the unique ability to support replication of primary and TCLA HIV-1 strains, allowing comparison of envelope glycoprotein functions against a common cellular background (2,12). MIP-1α, MIP-1ß and RANTES are most active against HIV-1 in combination (2,3), and strongly inhibited infection of PM1 cells by complemented viruses whose envelopes are derived from the NSI primary strains ADA and BaL (Table 1a).

Table 1: Inhibition of HIV-1 entry in PM1 cells and CD4⁺ T-cells by ß-chemokines

	% luciferase activity				
	BaL	**ADA**	**NL4/3**	**HxB2**	**MuLV**
a)					
PM1 cells					
control without virus	2	2	2	5	3
control with virus	100	100	100	100	100
+R/Mα/Mß (50/50/50)	2	3	92	117	100
+RANTES (100)	1	1	nd	nd	nd
+MIP-1α(100)	54	54	nd	nd	nd
+MIP-1ß (100)	1	6	nd	nd	nd
+MCP-1 (100)	46	50	nd	nd	nd
+MCP-2 (100)	28	26	nd	nd	nd
+MCP-3 (100)	58	46	nd	nd	nd
b)	**JR-FL**	**HxB2**	**MuLV**		
LW4 CD4⁺ T-cells					
control without virus	1	1	1		
control with virus	100	100	100		
+R/Mα/Mß (200/200/200)	14	68	nd		
LW5 CD4⁺ T-cells					
control without virus	1	1	1		
control with virus	100	100	100		
+R/Mα/Mß (200/200/200)	15	73	nd		

Table 1 legend:

PM1 cells were cultured as described by Lusso et al (12). Ficoll/hypaque-isolated PBMC from laboratory workers (LW) stimulated with PHA for 72h before depletion of CD8+ Lymphocytes with anti-CD8 immunomagnetic beads (DYNAL, Great Neck, NY). CD4+ Lymphocytes were maintained in culture medium containing interleukin-2 (100U/ml; Hofmann LaRoche, Nutley, NJ), as described previously (3). Target cells ($1-2x10^5$) were infected with supernatants (10-50ng of HIV-1 p24) from 293-cells co-transfected with an NL4/3Δenv-luciferase vector and a HIV-1 env-expressing vector (10,11). ß-Chemokines (R & D Systems, Minneapolis) were added to the target cells simultaneously with virus, at the final concentrations (ng/ml) indicated in parentheses in the first column. The ß-chemokine concentration range was selected based on prior studies (2,3). After 2h, the cells were washed twice with PBS, resuspended in ß-chemokine-containing media and maintained for 48-96h. Luciferase activity in cell lysates was measured as described previously (10,11). The values indicated represent luciferase activity (cpm)/ng p24/mg protein, expressed relative to that in virus-control cultures lacking ß-chemokines (100%), and are the means of duplicate or sextuplicate determinations. nd, not done. R/Mα/Mß, RANTES + MIP-1α + MIP-1ß.

RANTES and MIP-1ß were strongly active when added individually, while other ß-chemokines - MlP-1α, MCP-1, MCP-2 and MCP-3 (refs. 13-15) - were weaker inhibitors (Table 1a). However, MIP-1α, MIP-1ß and RANTES, in combination, did not inhibit infection of PM1 cells by the TCLA strains NL4/3 and HxB2, or by the amphotropic murine leukemia virus (MuLV-Ampho) pseudotype (Table 1a). Thus, phenotypic characteristics of the HIV-1 envelope

glycoproteins influence their sensitivity to ß-chemokines in a virus entry assay.

The *env*-complementation assay was used to assess HIV-1 entry into CD4+ T-cells from two control individuals (LW4 and LW5). MIP-1α, MIP-1ß and RANTES strongly inhibited infection by the NSI primary strain JR-FL infection of LW4's and LW5's $CD4^+$ T-cells, and weakly reduced HxB2 infection of LW cells (Table 1b), suggesting that there may be some overlap in receptor usage on activated $CD4^+$ T-cells by different virus strains. BaL env-mediated replication in normal PBL was also inhibited by MIP-1α, MIP-1ß and RANTES, albeit with significant inter-donor variation in sensitivity (data not shown).

It was determined when ß-chemokines inhibited HIV-1 replication by showing that complete inhibition of infection of PM1 cells required the continuous presence of ß-chemokines for up to 5h after addition of ADA or BaL env-complemented virus (Fig.1a). Pre-treatment of the cells with ß-chemokines for 2h or 24h prior to infection had no inhibitory effect if the cells were subsequently washed before virus addition. Furthermore, adding ß-chemokines 2h after virus only minimally affected virus entry (Fig.1a). A PCR-based assay was next used to detect HIV-1 early DNA reverse transcripts in PM1 cells after 10h of infection; reverse transcription of ADA, but not of NL4/3, could not be detected in the presence of MIP-1ß and RANTES (Fig.1b). Thus, inhibition by ß-chemokines requires their presence during at least one of the early stages of HIV-1 replication: virus attachment, fusion and early reverse transcription.

These sites of action were discriminated, first by testing whether ß-chemokines inhibited binding of JR-FL or BRU gp120

to soluble CD4, or of tetrameric CD4-IgG2 binding to HeLa-JR-FL cells expressing oligomeric envelope glycoproteins (17). No inhibition by any of the ß-chemokines was found in either assay, whereas the OKT4a CD4-MAb was strongly inhibitory in both (data not shown). Thus, ß-chemokines inhibit a step after CD4 binding, when conformational changes in the envelope glycoproteins lead to fusion of the viral and cellular membranes (18). Cell-cell membrane fusion is also induced by the gp120-CD4 interaction, and can be monitored directly by resonance energy transfer (RET) between fluorescent dyes incorporated into cell membranes (17). In the RET assay, OKT4a completely inhibits membrane fusion of PM1 cells with HeLa cells expressing the envelope glycoproteins of either JR-FL (HeLa-JR-FL) or BRU (HeLa-BRU), confirming the specificity of the process (17). RANTES, MIP-1ß (and to a lesser extent, MIP-1α) strongly inhibited membrane fusion of HeLa-JR-FL cells with PM1 cells, whereas fusion between PM1 cells and HeLa-BRU cells was insensitive to these ß-chemokines (Table 2a).

Table 2:Effect of ß-chemokines on HIV-1 envelope glycoprotein-mediated membrane fusion measured using the RET assay

	% Fusion	
	HeLa-JR-FL	HeLa-BRU
a) PM1 cells		
no chemokines	100	100
+R/Mα/Mß (80/400/100)	1	95
+RANTES (80)	8	100
+MIP-1α (400)	39	100
+MIP-1ß (100)	13	93
+MCP-1 (100)	99	98
+MCP-2 (100)	72	93
+MCP-3 (100)	98	99
b) LW5 $CD4^+$ cells		
no chemokines	100	100
+R/Mα/Mß(106/533/133)	39	100
+RANTES (106)	65	95
+MIP-1α (533)	72	100
+MIP-1ß (133)	44	92
+OKT4A (3ug/ml)	0	0

Table 2 legend:
$CD4^+$ target cells (mitogen-activated $CD4^+$ lymphocytes or PM1 cells) were labeled with octadecyl rhodamine (Molecular Probes, Eugene, OR), and HeLa-JR-FL cells, HeLa-BRU cells (or control HeLa cells, not shown) were labeled with octadecyl fluorescein (Molecular Probes), overnight at 37°C. Equal numbers of labeled target cells and env-expressing cells were mixed in 96-well plates and ß-chemokines (or CD4 MAb OKT4a) were added at the final concentrations (ng/ml) indicated in parentheses in the first column. Fluorescence emission values were determined 4h after cell mixing (17). If cell fusion occurs, the dyes are closely associated in the conjoined membrane such that excitation of fluorescein at 450nm results in resonance energy transfer (RET) and emission by rhodamine at 590nm. Percentage fusion is defined as equal to 100 x [(Exp RET - Min RET) / (Max RET - Min RET)], where Max RET = %RET obtained when HeLa-Env and $CD4^+$ cells are mixed, Exp RET = %RET obtained when HeLa-Env and

$CD4^+$ cells are mixed in the presence of fusion-inhibitory compounds, and Min RET = %RET obtained when HeLa cells (lacking HIV-1 envelope glycoproteins) and $CD4^+$ cells are mixed. The %RET value is defined by a calculation described elsewhere(17), and each is the mean of triplicate determinations. These values were, for HeLa-JR-FL and HeLa-BRU cells respectively: PM1 cells 11.5%, 10.5%; LW5 $CD4^+$ cells, 6.0%, 10.5%; R/Mα/Mß, RANTES + MIP-1α + MIP-1ß.

Similar results were obtained with primary $CD4^+$ T-cells from LW5 (Table 2b), although higher concentrations of ß-chemokines were required to inhibit membrane fusion in the primary cells than in PM1 cells. Thus, the actions of the ß-chemokines are not restricted to the PM1 cell line. The RET assay demonstrates that ß-chemokines interfere with env-mediated membrane fusion.

The simplest explanation of these results is that the binding of certain ß-chemokines to their receptor(s) prevents, directly or otherwise, the fusion of HIV-1 with $CD4^+$ T-cells. It has been known for a decade that HIV-1 requires a second receptor for entry into $CD4^+$ cells (19-21). This function is supplied, for TCLA strains, by fusin (9). Several receptors for MIP-1α, MIP-1ß and RANTES have been identified (6,7), and ß-chemokines exhibit considerable cross-reactivity in receptor usage (4-8). However, C-C CKR-1 and, especially, C-C CKR-5 were identified as the most likely candidates, based on tissue expression patterns and their abilities to bind MIP-1α, MIP-1ß and RANTES (4,7,8,15,22). C-C CKR-1, C-C CKR-5 and LESTR are each expressed at the mRNA level in PM1 cells and primary macrophages (data not shown). These and other ß-chemokine receptors were therefore PCR-amplified, cloned and expressed.

The expression of C-C CKR-5 in HeLa-CD4 (human), COS-CD4 (simian) and 3T3-CD4 (murine) cells rendered each of them readily infectible by the primary, NSI strains ADA and BaL in the env-complementation assay of HIV-1 entry (Table 3).

Table 3: C-C CKR-5 expression permits infection of CD4-expressing cells by primary, NSI HIV-1 strains

									R/Mα/Mß
		pcDNA3.1	LESTR	CKR-1	CKR-2a	CKR-3	CKR-4	CKR-5	CKR-5
COS-CD4	ADA	798	456	600	816	516	534	153000	3210
	BaL	660	378	600	636	516	618	58800	756
	HxB2	5800	96700	5240	5070	5470	5620	4850	5000
HeLa-CD4	ADA	678	558	4500	912	558	600	310000	6336
	BaL	630	738	1800	654	516	636	104000	750
	HxB2	337000	nd	nd	nd	nd	nd	nd	356000
3T3-CD4	ADA	468	558	450	618	534	606	28400	1220
	BaL	606	738	660	738	534	558	11700	756
	HxB2	456	24800	618	672	732	606	618	606

Table 3 legend:

Chemokine receptor genes C-C CKR-1, C-C CKR-2a, C-C CKR-3, C-C CKR-4 and C-C CKR-5 have no introns (4-8,15,22) and were isolated by PCR performed directly on a human genomic DNA pool derived from the PBMC of seven healthy donors. Oligonucleotides overlapping the ATG and the stop codons and containing BamHI and Xhol restriction sites for directional cloning into the pcDNA3.1 expression vector (Invitrogen Inc.) were used. LESTR (also known as fusin or HUMSTR) (4,9,24) was cloned by PCR performed directly on cDNA derived from PM1 cells, using sequences derived from the NIH database. Listed below are the 5'and 3' primer pairs used in first (5-1 and 3-1) and second (5-2 and 3-2) round PCR amplification of the CKR genes directly from human genomic DNA, and of LESTR from PM1 cDNA. Only a single set of primers was used to amplify CKR-5.

LESTR: L/5-1 = AAG CTT GGA GAA CCA GCG GTT ACC ATG GAG GGG ATC (SEQ ID NO: 9);

L/5-2 = GTC TGA GTC TGA GTC AAG CTT GGA GAA CCA (SEQ ID NO: 10);

L/3-1 = CTC GAG CAT CTG TGT TAG CTG GAG TGA AAA CTT GAA GAC TC (SEQ ID NO: 11);

L/3-2 = GTC TGA GTC TGA GTC CTC GAG CAT CTG TGT (SEQ ID NO: 12);

CKR-1:C1/5-1 = AAG CTT CAG AGA GAA GCC GGG ATG GAA ACT CC (SEQ ID NO: 13);

C1/5-2 = GTC TGA GTC TGA GTC AAG CTT CAG AGA GAA (SEQ ID NO: 14);

C1/3-1 = CTC GAG CTG AGT CAG AAC CCA GCA GAG AGT TC (SEQ ID NO: 15);

C1/3-2 = GTC TGA GTC TGA GTC CTC GAG CTG AGT CAG (SEQ ID NO: 16);

CKR-2a:C2/5-1 = AAG CTT CAG TAC ATC CAC AAC ATG CTG TCC AC (SEQ ID NO: 17);

C2/5-2= GTC TGA GTC TGA GTC AAG CTT CAG TAC ATC (SEQ ID

NO: 18);

C2/3-1 = CTC GAG CCT CGT TTT ATA AAC CAG CCG AGA C (SEQ ID NO: 19);

C2/3-2 = GTC TGA GTC TGA GTC CTC GAG CCT CGT TTT (SEQ ID NO: 20);

CKR-3: C3/5-1 = AAG CTT CAG GGA GAA GTG AAA TGA CAA CC (SEQ ID NO: 21);

C3/5-2= GTC TGA GTC TGA GTC AAG CTT CAG GGA GAA (SEQ ID NO: 22);

C3/3-1 = CTC GAG CAG ACC TAA AAC ACA ATA GAG AGT TCC (SEQ ID NO: 23);

C3/3-2 = GTC TGA GTC TGA GTC CTC GAG CAG ACC TAA (SEQ ID NO: 24);

CKR-4: C4/5-1 = AAG CTT CTG TAG AGT TAA AAA ATG AAC CCC ACG G (SEQ ID NO: 25);

C4/5-2 = GTC TGA GTC TGA GTC AAG CTT CTG TAG AGT (SEQ ID NO: 26);

C4/3-1 = CTC GAG CCA TTT CAT TTT TCT ACA GGA CAG CAT C (SEQ ID NO: 27);

C4/3-2 = GTC TGA GTC TGA GTC CTC GAG CCA TTT CAT (SEQ ID NO: 28);

CKR-5: C5/5-12 = GTC TGA GTC TGA GTC AAG CTT AAC AAG ATG GAT TAT CAA (SEQ ID NO: 29);

C5/3-12 = GTC TGA GTC TGA GTC CTC GAG TCC GTG TCA CAA GCC CAC (SEQ ID NO: 30).

The human CD4-expressing cell lines HeLa-CD4 (P42), 3T3-CD4 (sc6) and COS-CD4 (Z28T1) (23) were transfected with the different pcDNA3.1-CKR constructs by the calcium phosphate method, then infected 48h later with different reporter viruses (200ng of HIV-1 p24/10^6 cells) in the presence or absence of ß-chemokines (400ng/ml each of RANTES, MIP-1α and MIP-1ß). Luciferase activity in cell lysates was measured 48h later (10,11). ß-Chemokine blocking data is only shown for C-C CKR-5, as infection mediated by the other C-C CKR genes was too weak for inhibition to be quantifiable. In PCR-based assays of HIV-1 entry, a low level of entry of NL4/3 and ADA into

C-C CKR-1 expressing cells (data not shown) was consistently observed.

Neither LESTR nor C-C CKR-1, -2a, -3 or -4 could substitute for C-C CKR-5 in this assay. The expression of LESTR in COS-CD4 and 3T3-CD4 cells permitted HxB2 entry, and HxB2 readily entered untransfected (or control plasmid-transfected) HeLa-CD4 cells (Table 3). Entry of BAL and ADA into all three C-C CKR-5-expressing cell lines was almost completely inhibited by the combination of MIP-1α, MIP-1ß and RANTES, whereas HxB2 entry into LESTR-expressing cells was insensitive to ß chemokines (Table 3). These results suggest that C-C CKR-5 functions as a ß-chemokine-sensitive second receptor for primary, NSI HIV-1 strains.

The second receptor function of C-C CKR-5 was confirmed in assays of env-mediated membrane fusion. When C-C CKR-5 was transiently expressed in COS and HeLa cell lines that permanently expressed human CD4, both cell lines fused strongly with HeLa cells expressing the JR-FL envelope glycoproteins, whereas no fusion occurred when control plasmids were used (data not shown). Expression of LESTR instead of C-C CKR-5 did not permit either COS-CD4 or HeLa-CD4 cells to fuse with HeLa-JR-FL cells, but did allow fusion between COS-CD4 cells and HeLa-BRU cells (data not shown).

The fusion capacity of ß-chemokine receptors was also tested in the RET assay. The expression of C-C CKR-5, but not of C-C CKR-1, -2a, -3 or -4, permitted strong fusion between HeLa-CD4 cells and HeLa-JR-FL cells. The extent of fusion between HeLa-JR-FL cells and C-C CKR-5-expressing HeLa-CD4 cells was greater than the constitutive level of fusion between HeLa-BRU cells and HeLa-CD4 cells (Fig.2). The fusion-conferring function of C-C CKR-5 for primary, NSI HIV-1 strains has therefore

been confirmed in two independent fusion assays.

Experimental Discussion

Together, the above results establish that MIP-1α, MIP-1ß and RANTES inhibit HIV-1 infection at the entry stage, by interfering with the virus-cell fusion reaction subsequent to CD4 binding. It was also shown that C-C CKR-5 can serve as a second receptor for entry of primary NSI strains of HIV-1 into CD4+ T-cells, and that the interaction of ß-chemokines with C-C CKR-5 inhibits the HIV-1 fusion reaction.

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(vi) CURRENT APPLICATION DATA:
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(B) FILING DATE:
(C) CLASSIFICATION:

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(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 38 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

CAAGGCTACT TCCCTGATTG GCAGAACTAC ACACCAGG 38

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 base pairs

(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

AGCAAGCCGA GTCCTGCGTC GAGAG 25

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

GGGACTTTCC GCTGGGGACT TTC 23

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

CCTGTTCGGG CGCCACTGCT AGAGATTTTC CAC 33

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 31 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

Met Asp Tyr Gln Val Ser Ser Pro Ile Tyr Asp Ile Asn Tyr Tyr Thr
1 5 10 15

Ser Glu Pro Cys Gln Lys Ile Asn Val Lys Gln Ile Ala Ala Arg
20 25 30

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

His Tyr Ala Ala Ala Gln Trp Asp Phe Gly Asn Thr Met Cys Gln
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 32 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

Arg Ser Gln Lys Glu Gly Leu His Tyr Thr Cys Ser Ser His Phe Pro
1 5 10 15

Tyr Ser Gln Tyr Gln Phe Trp Lys Asn Phe Gln Thr Leu Lys Ile Val
20 25 30

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Gln Glu Phe Phe Gly Leu Asn Asn Cys Ser Ser Ser Asn Arg Leu Asp
1 5 10 15

Gln

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 36 base pairs
(B) TYPE: nucleic acid

(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

AAGCTTGGAG AACCAGCGGT TACCATGGAG GGGATC 36

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

GTCTGAGTCT GAGTCAAGCT TGGAGAACCA 30

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 41 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

CTCGAGCATC TGTGTTAGCT GGAGTGAAAA CTTGAAGACT C 41

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

GTCTGAGTCT GAGTCCTCGA GCATCTGTGT 30

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 32 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

AAGCTTCAGA GAGAAGCCGG GATGGAAACT CC 32

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

GTCTGAGTCT GAGTCAAGCT TCAGAGAGAA 30

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 32 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

CTCGAGCTGA GTCAGAACCC AGCAGAGAGT TC 32

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

GTCTGAGTCT GAGTCCTCGA GCTGAGTCAG 30

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 32 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

AAGCTTCAGT ACATCCACAA CATGCTGTCC AC 32

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

GTCTGAGTCT GAGTCAAGCT TCAGTACATC 30

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 31 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

CTCGAGCCTC GTTTTATAAA CCAGCCGAGA C 31

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

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(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 29 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

AAGCTTCAGG GAGAAGTGAA ATGACAACC 29

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

GTCTGAGTCT GAGTCAAGCT TCAGGGAGAA 30

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

CTCGAGCAGA CCTAAAACAC AATAGAGAGT TCC 33

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

GTCTGAGTCT GAGTCCTCGA GCAGACCTAA 30

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 34 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

AAGCTTCTGT AGAGTTAAAA AATGAACCCC ACGG 34

(2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

GTCTGAGTCT GAGTCAAGCT TCTGTAGAGT 30

(2) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 34 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

CTCGAGCCAT TTCATTTTTC TACAGGACAG CATC 34

(2) INFORMATION FOR SEQ ID NO:28:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

GTCTGAGTCT GAGTCCTCGA GCCATTTCAT 30

(2) INFORMATION FOR SEQ ID NO:29:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 39 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

GTCTGAGTCT GAGTCAAGCT TAACAAGATG GATTATCAA 39

(2) INFORMATION FOR SEQ ID NO:30:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 39 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

GTCTGAGTCT GAGTCCTCGA GTCCGTGTCG CAAGCCCAC 39

What is claimed is:

1. A polypeptide having a sequence corresponding to the sequence of a portion of a chemokine receptor and capable of inhibiting the fusion of HIV-1 to $CD4^+$ cells and thus of inhibiting HIV-1 infection of the cells.

2. The polypeptide of claim 1, wherein the chemokine receptor is C-C CKR-5.

3. The polypeptide of claim 2 comprising amino acids having a sequence of at least one extracellular domain of C-C CKR-5.

4. A pharmaceutical composition comprising an amount of the polypeptide of claim 1 effective to inhibit the fusion of HIV-1 to $CD4^+$ cells and a pharmaceutically acceptable carrier.

5. A polypeptide having a sequence corresponding to that of a portion of a HIV-1 envelope glycoprotein capable of specifically binding to the chemokine receptor C-C CKR-5.

6. A pharmaceutical composition comprising an effective amount of the polypeptide of claim 5 effective to inhibit the fusion of HIV-1 to $CD4^+$ cells and a pharmaceutically acceptable carrier.

7. An antibody or a portion of an antibody capable of binding to a chemokine receptor on a $CD4^+$ cell and inhibiting HIV-1 infection of the cell.

8. A pharmaceutical composition comprising an amount of the antibody of claim 7 effective to inhibit HIV-1 infection of $CD4^+$ cells and a pharmaceutically

acceptable carrier.

9. A method of treating an HIV-1 infected subject which comprises administering to the subject the polypeptide of any of claims 1, 2, 3, 5, or 7 in an amount effective to inhibit the fusion of HIV-1 to $CD4^+$ cells of the subject and thus treat the subject.

10. A method of reducing the likelihood of a subject from becoming infected by HIV-1 which comprises administering to the subject the polypeptide of any of claims 1, 2, 3, 5, or 7 in an amount effective to inhibit the fusion of HIV-1 to CD4+ cells of the subject and thus reduce the likelihood of HIV-1 infection.

11. A method for inhibiting HIV-1 infection of $CD4^+$ cells which comprises contacting such $CD4^+$ cells with a non-chemokine agent capable of binding to the chemokine receptor C-C CKR-5 in an amount and under conditions such that fusion of HIV-1 to the $CD4^+$ cells is inhibited, thereby inhibiting HIV-1 infection of the cells.

12. The method of claim 11, wherein the non-chemokine agent is an oligopeptide.

13. The method of claim 11, wherein the non-chemokine agent is a polypeptide.

14. The method of claim 11, wherein the non-chemokine agent is a nonpeptidyl agent.

15. A non-chemokine agent capable of binding to the chemokine receptor C-C CKR-5 and inhibiting the fusion of HIV-1 to $CD4^+$ cells.

16. A molecule capable of binding to the chemokine receptor C-C CKR-5 and inhibiting fusion of HIV-1 to $CD4^+$ cells comprising a non-chemokine agent linked to a ligand capable of binding to a cell surface receptor of the $CD4^+$ cells other than the chemokine receptor such that the binding of the non-chemokine agent to the chemokine receptor does not prevent the binding of the ligand to the other receptor.

17. The molecule of claim 16, wherein the cell surface receptor is CD4.

18. The molecule of claim 16, wherein the ligand comprises an antibody or a portion of an antibody.

19. A molecule capable of binding to the chemokine receptor C-C CKR-5 and inhibiting fusion of HIV-1 to $CD4^+$ cells comprising a non-chemokine agent linked to a compound capable of increasing the *in vivo* half-life of the non-chemokine agent.

20. The compound of claim 19, wherein the compound is polyethylene glycol.

21. A pharmaceutical composition comprising an amount of the molecule of claim 15, 16 or 19 effective to inhibit fusion of HIV-1 to $CD4^+$ cells and a pharmaceutically acceptable carrier.

22. A method for reducing the likelihood of HIV-1 infection in a subject comprising administering the pharmaceutical composition of claim 18 or 21 to the subject.

23. A method for treating HIV-1 infection in a subject comprising administering the pharmaceutical composition of claim 18 or 21 to the subject.

24. A method for determining whether a non-chemokine agent is capable of inhibiting the fusion of HIV-1 to a $CD4^+$, C-C $CKR-5^+$ cell which comprises:

(a) contacting the $CD4^+$, C-C $CKR-5^+$ cell, after it is labeled with a first dye, with a cell expressing an appropriate HIV-1 envelope glycoprotein on its surface, and labeled with a second dye, in the presence of an excess of the agent under conditions permitting fusion of the $CD4^+$, C-C $CKR-5^+$ cell to the cell expressing the HIV-1 envelope glycoprotein on its surface in the absence of an agent known to inhibit fusion of HIV-1 to $CD4^+$, C-C $CKR-5^+$ cells, the first and second dyes being selected so as to allow resonance energy transfer between the dyes;

(b) exposing the product of step (a) to conditions which would result in resonance energy transfer if fusion has occurred; and

(c) determining whether there is resonance energy transfer, the absence or reduction of transfer indicating that the agent is capable of inhibiting fusion of HIV-1 to $CD4^+$ and C-C $CKR-5^+$ cells.

25. The method of claim 24, wherein the agent is an oligopeptide.

26. The method of claim 24, wherein the agent is a polypeptide.

27. The method of claim 24, wherein the agent is a nonpeptidyl agent.

28. The method of claim 24, wherein the $CD4^+$ cell is a PM1 cell.

29. The method of claim 24, wherein the cell expressing the HIV-1 envelope glycoprotein is a HeLa cell expressing HIV-1_{JR-FL} gp120/gp41.

30. A transgenic nonhuman animal which comprises an isolated DNA molecule encoding the chemokine receptor C-C CKR-5.

31. The transgenic nonhuman animal of claim 30 further comprising an isolated DNA molecule encoding a sufficient portion of the CD4 molecule to permit binding the HIV-1 envelope glycoprotein.

32. A transgenic nonhuman animal which comprises an isolated DNA molecule encoding the chemokine receptor C-C CKR-5 and an isolated DNA molecule encoding fusin.

33. The transgenic nonhuman animal of claim 32 further comprising an isolated DNA molecule encoding a sufficient portion of the CD4 molecule to permit binding the HIV-1 envelope glycoprotein.

34. A transformed cell which comprises an isolated nucleic acid molecule encoding the chemokine receptor C-C CKR-5.

USES OF A CHEMOKINE RECEPTOR FOR INHIBITING HIV-1 INFECTION

Abstract of the Disclosure

This invention provides a polypeptide comprising a fragment of a chemokine receptor capable of inhibiting HIV-1 infection. In an embodiment, the chemokine receptor is C-C CKR-5. In another embodiment, the fragment comprises at least one extracellular domain of the chemokine receptor C-C CKR-5. This invention further provides different uses of the chemokine receptor for inhibiting HIV-1 infection.

What is claimed is:

1. A method for inhibiting fusion of HIV-1 to $CD4^+$ cells which comprises contacting $CD4^+$ cells with a non-chemokine agent capable of binding to a chemokine receptor in an amount and under conditions such that fusion of HIV-1 to the $CD4^+$ cells is inhibited.

2. A method for inhibiting HIV-1 infection of $CD4^+$ cells which comprises contacting $CD4^+$ cells with a non-chemokine agent capable of binding to a chemokine receptor in an amount and under conditions such that fusion of HIV-1 to the $CD4^+$ cells is inhibited, thereby inhibiting HIV-1 infection.

3. The method of claim 1 or 2, wherein the non-chemokine agent is an oligopeptide.

4. The method of claim 1 or 2, wherein the non-chemokine agent is a polypeptide.

5. The method of claim 1 or 2, wherein the non-chemokine agent is an antibody or a portion of an antibody.

6. The method of claim 1 or 2, wherein the non-chemokine agent is a nonpeptidyl agent.

7. A non-chemokine agent capable of binding to a chemokine receptor and inhibiting fusion of HIV-1 to $CD4^+$ cells.

8. The non-chemokine agent of claim 7, wherein the non-chemokine agent is a polypeptide.

9. The non-chemokine agent of claim 8, wherein the polypeptide is as set forth in SEQ ID NO:5.

10. An agent capable of binding to fusin and inhibiting HIV-1 infection.

11. The agent of claim 10, wherein the agent is an oligopeptide.

12. The agent of claim 10, wherein the agent is an polypeptide.

13. The agent of claim 10, wherein the agent is an antibody or a portion of an antibody.

14. The agent of claim 10, wherein the agent is a nonypeptidyl agent.

15. A pharmaceutical composition comprising an amount of the non-chemokine agent of claim 7 or 10 effective to inhibit fusion of HIV-1 to $CD4^+$ cells and a pharmaceutically acceptable carrier.

16. A composition of matter capable of binding to a chemokine receptor and inhibiting fusion of HIV-1 to $CD4^+$ cells comprising a non-chemokine agent linked to a ligand capable of binding to a cell surface receptor of the $CD4^+$ cells other than the chemokine receptor such that the binding of the non-chemokine agent to the chemokine receptor does not inhibit the binding of the ligand to the other receptor.

17. The composition of matter of claim 16, wherein the cell surface receptor is CD4.

18. The composition of matter of claim 16, wherein the ligand comprises an antibody or a portion of an antibody.

19. A pharmaceutical composition comprising an amount of the composition of matter of claim 16 effective to inhibit fusion of HIV-1 to $CD4^+$ cells and a pharmaceutically acceptable carrier.

20. A composition of matter capable of binding to the chemokine receptor and inhibiting fusion of HIV-1 to $CD4^+$ cells comprising a non-chemokine agent linked to a compound capable of increasing the *in vivo* half-life of the non-chemokine agent.

21. The composition of matter of claim 20, wherein the compound is polyethylene glycol.

22. A pharmaceutical composition comprising an amount of the composition of claim 20 effective to inhibit fusion of HIV-1 to $CD4^+$ cells and a pharmaceutically acceptable carrier.

23. A method for reducing the likelihood of HIV-1 infection in a subject comprising administering the pharmaceutical composition of claim 15, 19 or 22 to the subject.

24. A method for treating HIV-1 infection in a subject comprising administering the pharmaceutical composition of claim 15, 19 or 22 to the subject.

25. A method for determining whether a non-chemokine agent is capable of inhibiting the fusion of HIV-1 to a $CD4^+$ cell which comprises:

(a) contacting (i) a $CD4^+$ cell, which is labeled with a first dye, with (ii) a cell expressing the HIV-1 envelope glycoprotein on its surface, which is labeled with a second dye, in the presence of an

excess of the agent under conditions permitting the fusion of the $CD4^+$ cell to the cell expressing the HIV-1 envelope glycoprotein on its surface in the absence of the agent, the first and second dyes being selected so as to allow resonance energy transfer between the dyes;

(b) exposing the product of step (a) to conditions which would result in resonance energy transfer if fusion has occurred; and

(c) determining whether there is a reduction of resonance energy transfer, when compared with the resonance energy transfer in the absence of the agent, a decrease in transfer indicating that the agent is capable of inhibiting fusion of HIV-1 to $CD4^+$ cells.

26. The method of claim 25, wherein the agent is an oligopeptide.

27. The method of claim 25, wherein the agent is a polypeptide.

28. The method of claim 25, wherein the agent is an antibody or a portion of an antibody.

29. The method of claim 25, wherein the agent is a nonpeptidyl agent.

30. The method of claim 25, wherein the $CD4^+$ cell is a PM1 cell.

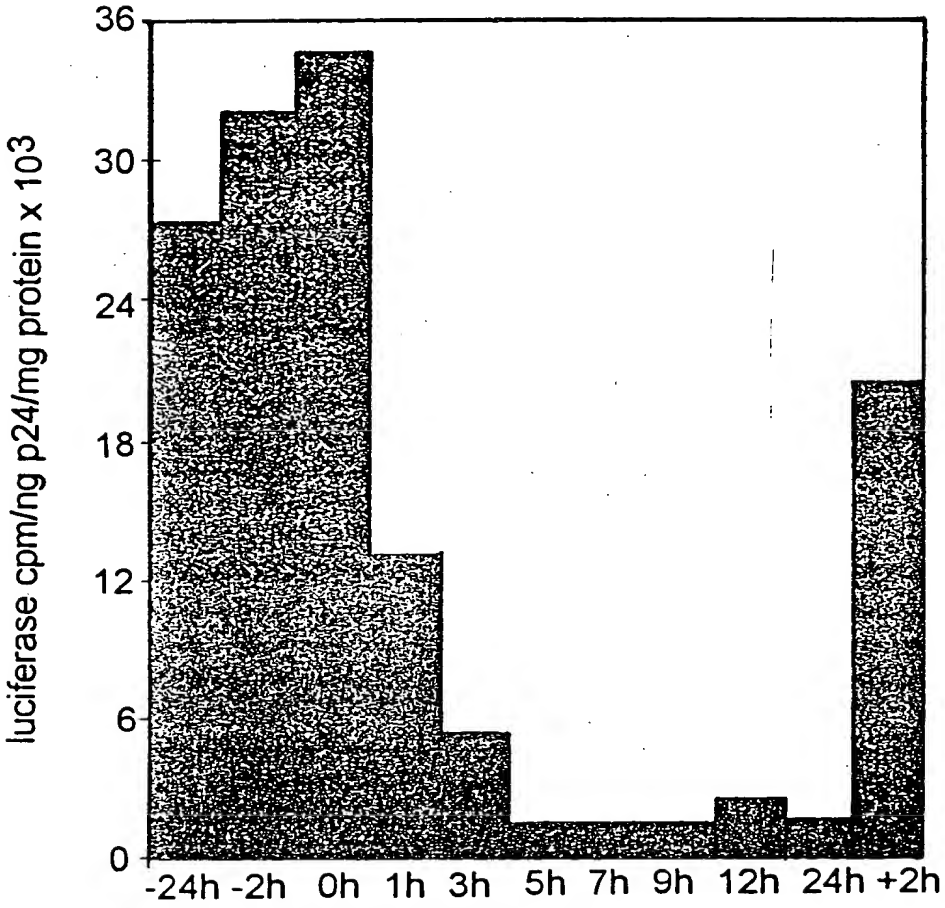
31. The method of claim 25, wherein the cell expressing the HIV-1 envelope glycoprotein is a HeLa cell expressing $HIV\text{-}1_{JR\text{-}FL}$ gp120/gp41.

A METHOD FOR PREVENTING HIV-1 INFECTION OF CD4$^+$ CELLS

Abstract of the Disclosure

This invention provides methods for inhibiting fusion of HIV-1 to CD4$^+$ cells which comprise contacting CD4$^+$ cells with a non-chemokine agent capable of binding to a chemokine receptor in an amount and under conditions such that fusion of HIV-1 to the CD4$^+$ cells is inhibited. This invention also provides methods for inhibiting HIV-1 infection of CD4$^+$ cells which comprise contacting CD4$^+$ cells with a non-chemokine agent capable of binding to a chemokine receptor in an amount and under conditions such that fusion of HIV-1 to the CD4$^+$ cells is inhibited, thereby inhibiting the HIV-1 infection. This invention provides non-chemokine agents capable of binding to the chemokine receptor and inhibiting fusion of HIV-1 to CD4$^+$ cells. This invention also provides pharmaceutical compositions comprising an amount of the non-chemokine agent capable of binding to the chemokine receptor and inhibiting fusion of HIV-1 to CD4$^+$ cells effective to prevent fusion of HIV-1 to CD4$^+$ cells and a pharmaceutically acceptable carrier.

FIG. 1A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30
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24h
+2h

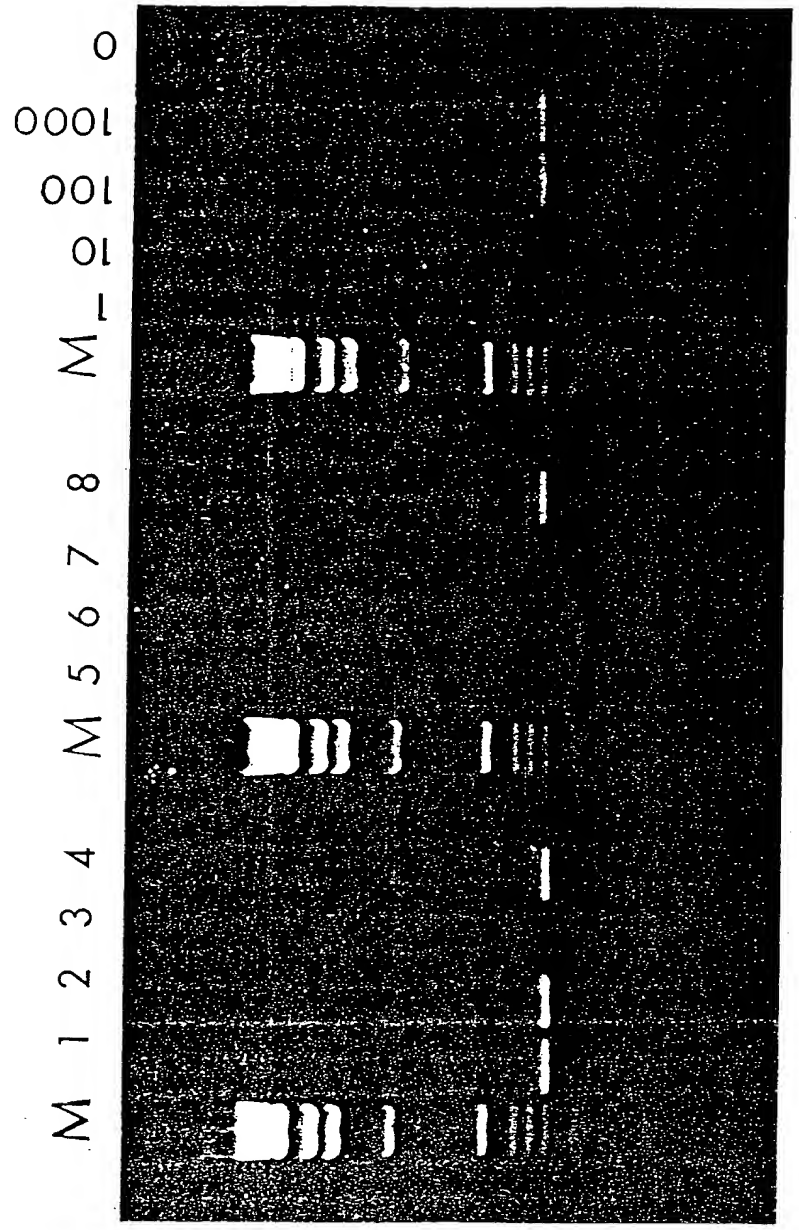
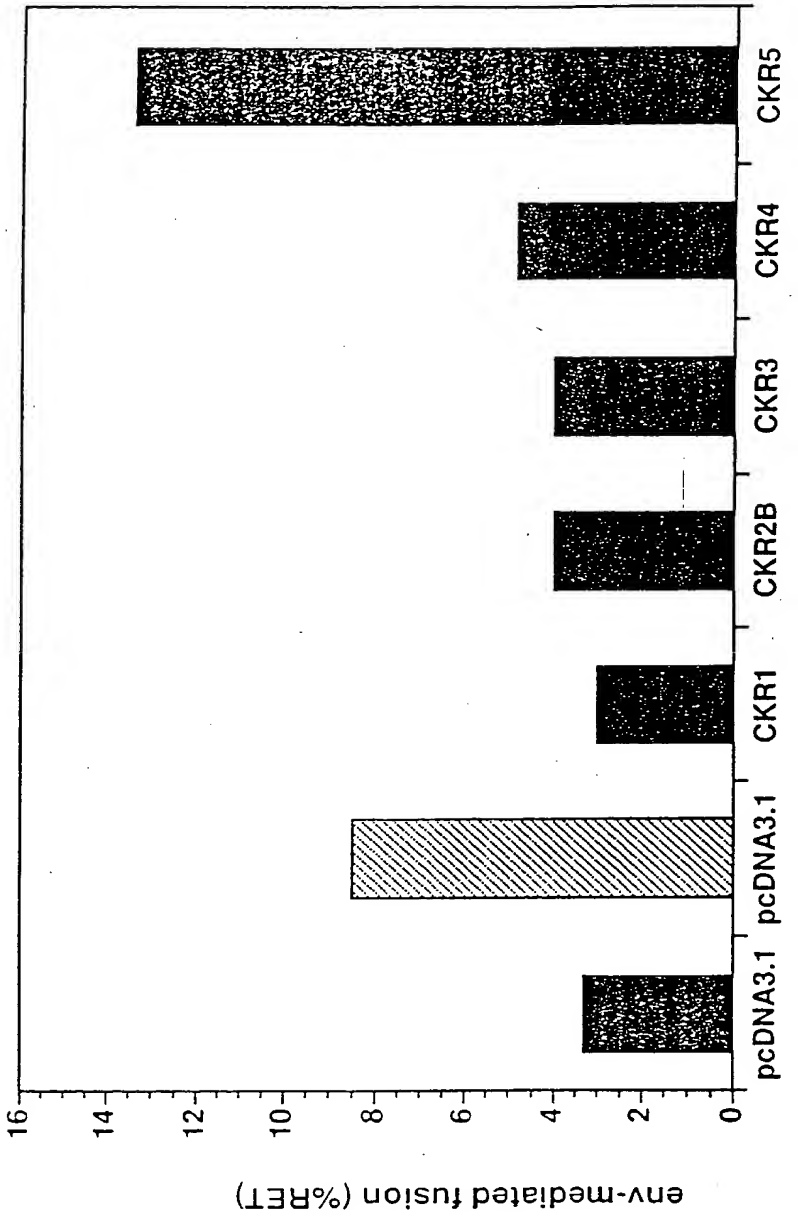


FIG. 1B

FIG. 2


env-mediated fusion (%RET)
0
2
4
6
8
10
12
14
16
pcDNA3.1
pcDNA3.1
CKR1
CKR2B
CKR3
CKR4
CKR5